PROTEOMIC SCREENING FOR LYSOSOMAL STORAGE DISEASES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/002,992 filed on Mar. 31, 2020, which is incorporated herein by reference in its entirety as if fully set forth herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under HD098180 and A1123135 awarded by the National Institutes of Health. The government has certain rights in the invention.

STATEMENT REGARDING SEQUENCE LISTING

[0003] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 2GU5154_ST25.txt. The text file is 74 KB, was created on Mar. 31, 2021, and is being submitted electronically via EFS-Web.

FIELD OF THE DISCLOSURE

[0004] The current disclosure provides clinical diagnosis and newborn screening for lysosomal storage diseases (LSDs) including Mucopolysaccharidosis Type I (MPS I or Hurler Syndrome) and Pompe Disease. The disclosed methods and assays can also allow rapid prediction of whether a patient with LSD will develop an immune response to enzyme replacement therapy (ERT), thus improving treatment for patients with LSDs. The disclosed methods and assays can further reduce the number of false positives caused by pseudo deficiency cases of LSD.

BACKGROUND OF THE DISCLOSURE

[0005] There are a number of diseases with effective treatments available. However, for a number of these diseases, once symptoms emerge, the disease is already fatal or has led to irreversible damage. Examples of such disorders include metabolic disorders, such as lysosomal storage diseases (LSDs). These include Mucopolysaccharidosis Type I (MPS I) and Pompe Disease.

[0006] LSDs include a group of more than 50 rare inherited metabolic disorders that result from defects in lysosome function. Lysosomes are intracellular compartments filled with enzymes responsible for the breakdown of large molecules and the relay of the breakdown fragments to other parts of the cell for recycling. This process requires several critical enzymes, and defects in one or more of these enzymes can cause the large molecules to accumulate within the cell, eventually killing the cell. Patients having an LSD can have damage to skeletal muscle, bones, and the nervous system.

[0007] Treatment for LSDs include providing functioning exogenous enzymes (e.g., in a form of a drug) in enzyme replacement therapy (ERT). However, some patients will develop immune-mediated inhibitory reactions, including neutralizing antibodies, to ERT. Immunomodulation can be undertaken to combat this immune response in a patient but is most effective when initiated prior to ERT. Therefore, knowing whether a patient suffering from an LSD will develop an immune reaction to ERT before starting the treatment can be critical. Currently, molecular analyses to predict which patients will develop such immune reactions are slow and labor-intensive, taking months to complete. During this time, patients may have developed ERT-neutralizing antibodies. Nonetheless, currently there are no standard clinical tests with fast turn-around times that can reliably help predict immune reactions to ERT.

[0008] Newborn screening (NBS) is a standard public preventive mandatory screening test carried out routinely for the 4 million babies born every year in the U.S. NBS usually involves a blood test performed 24 to 48 hours after birth. The screening usually uses a few drops of blood from a newborn's heel deposited on filter paper. The paper containing dried blood spots (DBS) can be stored until the tests are conducted.

[0009] To conduct NBS assessments, punches of dried blood are taken from the DBS and laboratory tests are performed to detect the presence or absence of specific substances within the blood (called markers or biomarkers) that are indicative of disorders not apparent at birth but that cause serious health problems later in life. Though the disorders screened vary from state to state, most states screen for phenylketonuria, primary congenital hypothyroidism, cystic fibrosis, and sickle cell disease. NBS has proven to be highly effective at improving patient outcomes and avoiding long-term disability in affected individuals, while at the same time reducing healthcare costs.

[0010] NBS for several LSDs, including MPS I and Pompe Disease, has been approved in many states. The screening involves measurement of lysosomal enzymatic activities in DBSs, typically by tandem mass spectrometry or digital microfluidics fluorimetry. Newborns having an assay value for enzyme activity below a predetermined cut-off value are considered positive for an LSD. However, lack of analytical precision can warrant additional second-tier tests to confirm screen-positive results. The enzymatic assay relies on synthetic substrates, which are not identical to the natural substrates; thus, the enzymes will behave differently towards those artificial substrates and potentially cause misdiagnosis. Furthermore, the enzymatic assay requires that the functions and structures of the relevant enzymes remain intact, which will be difficult to control during transportation and storage of the NBS samples from various parts of the country or states.

[0011] Therefore, robust and simple methods and assays are needed to screen for LSDs with lower false positive rate and higher positive prediction rate and simultaneously allow for rapid prediction of whether a patient will develop immune reactions to ERT.

SUMMARY OF THE DISCLOSURE

[0012] The current disclosure describes development of multiplexed assays that can be used to screen subjects for LSDs including Mucopolysaccharidosis Type I (MPS I; Hurler Syndrome) and Pompe Disease. The assays can significantly improve outcomes for affected individuals by reliably diagnosing these disorders before devastating and often fatal clinical symptoms emerge. The assays can detect the presence or absence of markers associated with these